weekly intra-articular injections (Artz, Seikagaku Corporation, Tokyo, Japan) after drainage of synovial fluid in every cases. We checked total volume of synovial fluid (SF) and measured: HA, C4S, C6S, KS and IL-6 before first and fifth time. Viscosity measurement was performed by using rotational viscometers. We excluded the case if synovial fluid could not be extracted. We decided not to administer aspirin and nonsteroidal anti-inflammatory drugs six weeks before injections and during injections. The range of motion was measured and clinical symptoms were using American Knee Society (AKS) knee and function scores. All knees were classified using the Kellgren-Lawrence grades (KL grade), and the femorotibial angle (FTA) was measured on the radiogram.

Results: Eighteen patients (18 knees) were not extracted before finished 5 weekly injections, they were excluded. Forty three knees (right 23 and left 20 knees) were examined. Mean age was 66 ± 9.5 years old, and mean body mass index was 23.7 ± 2.6 kg/m‡. KL grades were grade 1 in 3 knees, grade 2 in 24 knees, grade 3 in 14 knees and grade 4 in 2 knees. Mean FTA was 178.8 ± 3.6°. We didn't have complications and side effects. The volume of SF, which was 14.2ml before first injection and 7.3 ml before fifth injection, significantly decreased after HA injections ($p < 0.05$). Mean AKS knee score, which was 61 points before first injection and 77 points before fifth injection significantly improved after HA injections ($p < 0.05$). Mean AKS function score, which was 75 points before first injection and 91 points before fifth injection, significantly improved after HA injections ($p < 0.05$). Mean ROM, which was 129 ± 10° before first injection and 137 ± 7° before fifth injection, significantly improved after HA injections ($p < 0.05$). Mean HA level was significantly increased from 1.50 mg/ml to 1.72 mg/ml ($P < 0.05$). Mean C4S and C6S level were significantly decreased from 17.5 nmol/ml to 16.0 nmol/ml and from 59.7 nmol/ml to 50.2 nmol/ml, respectively ($P < 0.05$). Mean KS level was not significantly decreased from 6.62 μg/ml to 6.23 μg/ml. Mean IL-6 level was significantly decreased from 3733 pg/ml to 457 pg/ml ($P < 0.05$). The mean viscosity was significantly increased from 49.6 mPa S to 72.5 mPa S ($P < 0.05$).

Conclusions: The increase of HA and viscosity showed the nature of synovial fluid was improved. The decrease of C6S and C4S showed the destruction of proteoglycan in articular cartilage was inhibited. The decrease of IL-6 showed the inflammation of synovium was suppressed. This study was first report to indicate that IL-6 was decreased after intra-articular HA. We demonstrated changes of the biomarkers, the improvement of clinical scores and efficacy of intra-articular HA.

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MATERIAL ON THE BASIS OF PARTIALLY CROSSLINKED SOLUTION OF HA DERIVATIVE FOR SYNOVIAL FLUID VISCOSUPPLEMENTATION

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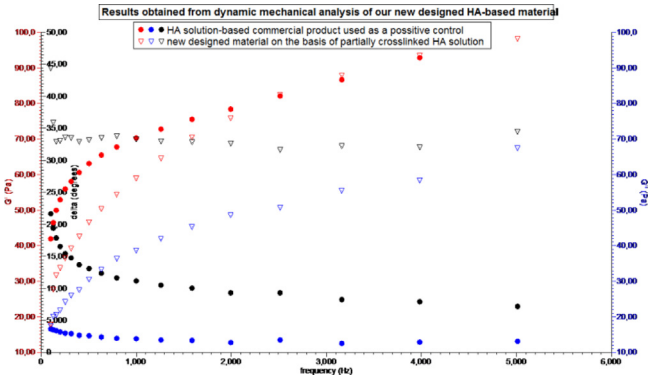
Purpose: Synovial fluid is a joint lubricant and plays an important role in cartilage deformation caused by mechanical load. The main component of synovial fluid is hyaluronic acid with an approximate concentration of 2,5 mg/ml and the average molecular weight of 3-5 MDa in healthy joints. It is known, that during osteoarthritis the concentration and the average molecular weight of HA in synovial fluid are significantly reduced. This has a negative effect on the viscoelasticity of the synovial fluid, which leads to significant decrease of the mechanical protection of the articular system. Also, the articular cartilage function deteriorates. It is also known that preparations with lower average molecular weight used in treatment have not sufficient viscoelastic properties and undergo biodegradation too quickly. In our previous work we designed a new HA tyramine derivative for the preparation of non-cytotoxic and biocompatible HA hydrogel-based materials with enhanced mechanical properties. This HA derivative was further optimized to become a material with suitable properties for synovial fluid viscosupplementation. For this purpose, partially crosslinked solutions with prolonged biostability compared to HA solution-based treatment preparations, were designed. In this work we would like to present viscoelastic properties of this material and results from *in vivo* tests, in which this material was evaluated in treatment of experimentally induced osteoarthritis in rabbit knee.

Methods: In this study viscoelastic properties of new designed partially crosslinked HA-based solutions were determined and optimized to reach the appropriate properties corresponding with synovial fluid. The viscoelastic properties were measured by dynamic mechanical analysis



in a frequency sweep oscillation mode (frequency 0, 1-10 Hz at 0, 5% strain) and by determination of storage modulus G′, loss modulus G″ and loss angle δ. After that the treatment effect of our new designed material was tested in rabbit knee. Experimental osteoarthritis was induced in male rabbit outbred line P91 by intraarticular injection of collagenase. The rabbits were intraarticularly injected with our material immediately after the osteoarthritis induction and then repeatedly once a week in the course of 5 weeks. The volume of the intraarticularly injected substance was 0, 3 ml. Animals treated with physiological solution as control and animals treated with HA solution-based commercial product as positive controls were also included in the tests. After 8 weeks, the rabbit cartilages were macromorphometrically evaluated. The cartilages were postmortem inspected and gross morphological changes of the medial and lateral condyles were rated according to the criteria published by Yoshioka *et al.*[1, 2]: grade 1 - intact surface; grade 2 - slightly rough surface, minimal fibrillation and erosions; grade 3 - markedly rough surface, fibrillation and hypertrophic cartilage with moderate erosions; grade 4 - severe erosions, loss of cartilage exposing the underlying bone. Histological evaluation was also performed.

Results:

Fig.1: Representative results obtained from Dynamic mechanical analysis in a frequency sweep oscillation mode (frequency 0, 1-10 Hz at 0, 5% strain) of our new designed HA-based material and commercial product used as a positive control

Fig.2: Results obtained from the evaluation of morphological changes in OA rabbit knee rated according the criteria mentioned above

Conclusions: The results obtained so far showed that our new designed material has suitable properties for synovial fluid viscosupplementation. Thanks to the partial crosslinking of the HA solutions, our material has enhanced viscoelastic properties and prolonged biostability compared to HA solution-based treatment preparations with insufficient molecular weight. The results from *in vivo* tests showed that the intraarticular injections of our material prevent the development of joint damage in the rabbit model of osteoarthritis. The morphological changes demonstrate a substantial decrease in severity of the damage caused in condyle cartilage.